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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Brines et al. Confirmation No.: 6595

Application No.: 09/716,960 Group Art Unit: 1647

Filed: November 21, 2000 Examiner: DeBerry, Regina M.

For: METHODS FOR PREVENTION AND TREATMENT OF NEURODEGENERATIVE CONDITIONS BY PERIPHERALLY ADMINISTERED ERYTHROPOIETIN (as amended)

Attorney Docket No.: 10165-009-999

DECLARATION OF MICHAEL L. BRINES, M.D., PH.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, MICHAEL L. BRINES, do hereby declare and state:

1. I am an inventor of the invention described and claimed in the above-identified patent application (hereinafter the " '960 application"). I am presently a scientist at The Kenneth S. Warren Institute, the assignee of the '960 application.

2. I have over twenty-five years of experience in the field of clinical investigation, particularly in the area of neurobiology and endocrinology research. My academic and technical experience and honors, and a list of my publications, are set forth in my curriculum vitae, a copy of which is attached hereto as Appendix 1.

3. I have read and am familiar with the '960 application, and the pending claims and the outstanding Office Action. I have been informed and believe that the claims of the '960 application are subject to a rejection based on the contention that the '960

application does not provide sufficient guidance for the use of recombinant EPO for preventing neurodegenerative disorders, and that such preventative methods would require undue experimentation.

4. The following experiments were conducted by me or under my supervision at The Kenneth S. Warren Institute. The experiments describe successful experiments in which recombinant EPO was used to protect against the development of symptoms of neurodegenerative disease in two distinct animal models of neurodegenerative disorders: a rat diabetic neuropathy model and a transgenic mice model ("the SOD model"), in which mice carry a mutated (G93A) human copper-zinc superoxide dismutase, an animal model of human ALS (amyotropic lateral sclerosis). As further evidence of the validity of these animal models as models for human neurodegenerative disorders, I have attached hereto, as part of Appendix 6, a number of peer-reviewed scientific articles that rely on these models for studies of a variety of neurodegenerative conditions.

5. The first model used to study the protective effect of EPO was a rat model of experimental diabetic neuropathy. Diabetes leads to a wide range of peripheral neuronal deficits, including reduced sensory and motor nerve conduction velocity, impaired sciatic nerve regeneration, axonal shrinkage in association with reduced neurofilament delivery and deficient anterograde axonal transport. In humans, neuropathy is one of the major complications contributing to morbidity in patients with diabetes mellitus. Painful diabetic neuropathy is associated with burning, tactile hypersensitivity and is treatable, symptomatically and specifically, with antidepressants, anticonvulsants, capsaicin, nonsteroidal anti-inflammatory drugs, opioids and aldose reductase inhibitors. However, such analgesic relief is often inadequate, potentially toxic, or is associated with dependence liability.

6. Streptozotocin ("STZ")-induced diabetes in rats is an art-accepted model of painful neuropathy. The streptozotocin-induced diabetic rats have been widely used as a model of insulin-dependent diabetes mellitus, and a number of anomalies similar to those observed in human diabetic complications have been demonstrated in this model. In particular, mechanical and thermal alteration of nociceptive thresholds has been observed following streptozotocin treatment. A single dose of streptozotocin leads to the development

of an abnormal pain syndrome in rats similar to that seen in patients with painful diabetic neuropathy.

7. The first set of experiments demonstrates the protective effect of chronic EPO treatment in preventing functional, structural and biochemical disorders of peripheral nerve in the streptozotocin-induced diabetes in rats. The effect of diabetes and EPO treatment on growth, water and food intake was investigated in diabetic animals. The animals suffered from the usual symptoms of this model. Glycosuria was elevated within two days, and remained elevated for the duration of the experiment, and was chosen as criteria to assign rats to the diabetic groups. A control group (non-diabetic) rats were treated, similarly to diabetic ones, with EPO.

8. As shown in Figure 1A, the growth rate was profoundly reduced in untreated and EPO-treated diabetic rats, with virtually no weight gain over the course of the experimental period. As shown in Figures 1B and 1C, animals displayed polyuria with a marked polydipsia and polyphagia.

9. Next, the effect of diabetes and EPO treatment on plasma glucose, hematocrit, and muscle weight was examined. After 7 weeks of diabetes, glycemia and hematocrit was measured in blood from tail vein. As shown in Table l, diabetic rats were hyperglycaemic, and EPO treatment did not significantly change blood glucose levels. The hematocrit was monitored in all the animals as shown in Table 2. After seven week diabetes, both body weight and weight of extensor digitorum longus (EDL) and soleus muscles were lower than age matched controls, indicating that muscle wasting contributed to the general weight loss (see Figure 1A and Table 1). EPO treatment did not have a significant effect on these parameters.

10. Figure 2 demonstrates the effect of EPO treatment on rat nociceptive threshold in diabetic rats. As shown in Figure 2, diabetic rats display a significant early increase in thermal threshold up to 4-5 weeks, rather than showing a thermal hyperalgesia (see Malcangio and Tomlinson, 1998). Hind paw thermal response latencies were significantly increased (by about 60%) in untreated diabetic rats compared with untreated controls at all time points tested. EPO had no effect on thermal response latencies in control rats, but significantly prevented the increased response latency in the hindpaw of diabetic rats

(to about 40%). At 4 and 5 weeks, EPO-treated group response latencies are significantly lower than in the untreated diabetic group (the reduction is 15-20%).

11. Figure 3 demonstrates the effect of EPO treatment on mechanical force-withdrawal threshold in diabetic rats. A single STZ injection on rat mechanical nociceptive threshold was monitored for 6.5 weeks after STZ injection by the paw pressure test according to Randall-Selitto. The force-withdrawal threshold in diabetic rats after treatment with EPO was significantly lower at all time points. EPO treatment for 4.5 and 5.5 weeks significantly decreased STZ-induced hyperalgesia ($p<0.05$).

12. The results on nerve conduction velocity are given in Table 3. Both STZ and STZ+ EPO groups are significantly different ($P<0.001$) from the control group. STZ+EPO group is significantly different ($P<0.01$) from the STZ group.

13. In summary, the results provided above demonstrate that EPO provides a protective effect for a number of neurodegerative symptoms and conditions in an experimental diabetic neuropathy model in the rat.

14. The next model used to study the protective effect of EPO was the SOD model, a transgenic mice model in which mice carry a mutated (G93A) human copper-zinc superoxide dismutase. The SOD model is an art-accepted animal model of human ALS. SOD mice become paralyzed as a result of motor neuron loss from the spinal cord and die at 5 to 6 months of age. Peer-reviewed scientific references which discuss this model in more detail are provided herewith as Appendix 6 (see Barnéoud and Curet, 1999, Experimental Neurology 155: 243-251; Bendotti *et al.*., 2001, J. Neurochemistry 79: 737-746; and Bordet *et al.*, 2001, Human Mol. Genetics 10: 1925-1933).

15. EPO was administered daily to the SOD mice, and the symptoms of neurodegeneration were compared in the EPO-treated mice and mice treated with vehicle (saline). As shown in Figure 4 panels A-D, treatment with EPO reduced the severity of a variety of neurodegenerative symptoms in these mice, as compared to mice treated with the vehicle. In particular, Figure 4, panel A, shows that EPO treatment did not modify weight gain in these mice, but reduced dramatic weight loss seen in control mice after 15 weeks. The results shown in Figure 4, panel B, demonstrate that EPO treatment did not significantly

modify stride length until 17 weeks; after 17 weeks, EPO-treated SOD mice decreased stride length at a significantly lower rate than control mice.

16. Motor coordination and performance was estimated in EPO-treated and untreated SOD mice by an extension reflex deficit test, the results of which are shown in Figure 4, panel C, and a rotarod test, shown in Figure 4, panel D. EPO-treated mice demonstrated reduced symptoms of degeneration and delayed time of onset of the loss of motor ability using both measures of motor performance.

17. The foregoing experiments are representative of the ability of EPO to protect against and alleviate the symptoms of neurodegenerative diseases. It is well-recognized with the field of neurobiology and endocrinology research that there are common conditions and mechanisms amongst various neurodegenerative disorders. In particular, it has been noted that, irrespective of the primary causes of individual neurodegenerative diseases, it is the onset of oxidative stress resulting from free radical mechanisms that results in neuronal cell death and progression of the disease (*see* Jenner, 1996, Pathol. Biol. (Paris) 44(1): 57-64). For example, biochemical changes that occur with Alzheimer's disease, motor neuron diseases and diabetic neuropathy suggest the role of oxidative stress in these diseases. *See id.* It has also been noted that changes in serum insulin, IGF-1, and/or IGF-binding protein are common to neurodegenerative diseases as well as disruptions in neuro-filament metabolism. *See, e.g.,* Busiguina *et al.*, 2000, Neurobiology of Disease 7: 657-665, at 663, last paragraph ("In summary, our results indicate that changes in serum insulin, IGF-I, and/or IGF-binding proteins are associated to many different types of neurodegenerative processes.") and Brownlees *et al.*, 2002, Human Molecular Genetics 11 (23) 2837-2844, at 2839-2840 ("An increasing body of evidence suggests that disruptions to neurofilament metabolism are part of the disease process in a number of neurodegenerative diseases."), which are attached hereto as part of Appendix 6.

18. The results and scientific references discussed hereinabove support the disclosure and claims of the '960 application which encompass methods for the prevention of neurodegenerative conditions by treatment with EPO. Taken together, these references and studies demonstrate that EPO can be used to protect against symptoms of a broad range of neurodegenerative conditions in two art-accepted models of neurodegenerative diseases.

19. I declare further that all statements made in this Declaration of my own knowledge are true, that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

February 26, 2003
Date: February 26, 2003

Michael L. Brines.
Michael L. Brines

Attachments:

Appendix 1: Curriculum Vitae of Dr. Michael L. Brines, Ph.D., M.D.;
Appendix 2: Experimental Protocols
Appendix 3: Tables 1-3
Appendix 4: Figures 1-3: Diabetic Neuropathy Model
Appendix 5: Figure: 4 SOD Model
Appendix 6: References on Neurodegenerative Conditions and Models